

Development and Application of a Solid Phase Extraction and Micro-Liquid Chromatography-Electrospray/Ion Trap Mass Spectrometry Method for Detecting Pharmaceuticals in Natural Waters

T.L. Jones-Lepp, K.E. Varner, A. Mitchell#*

U.S. Environmental Protection Agency

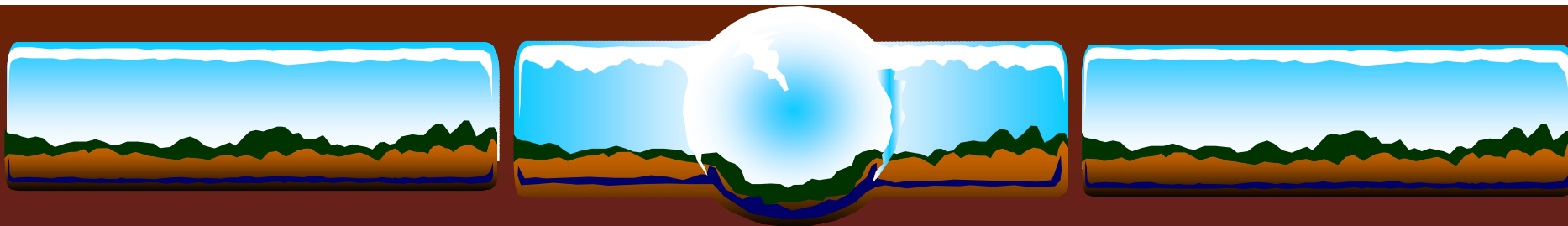
National Exposure Research Laboratory

Environmental Sciences Division

P.O. Box 93478, Las Vegas, NV 89193

#Morgan State University, Baltimore, MD

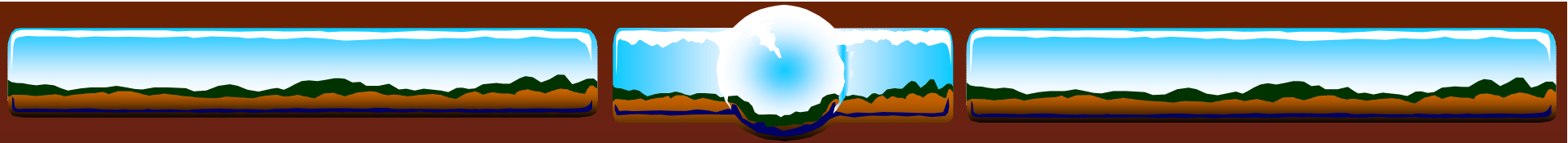




Development and Application of a Solid Phase Extraction and Micro-Liquid Chromatography-Electrospray/Ion trap Mass Spectrometry Method for Detecting Pharmaceuticals in Natural Waters

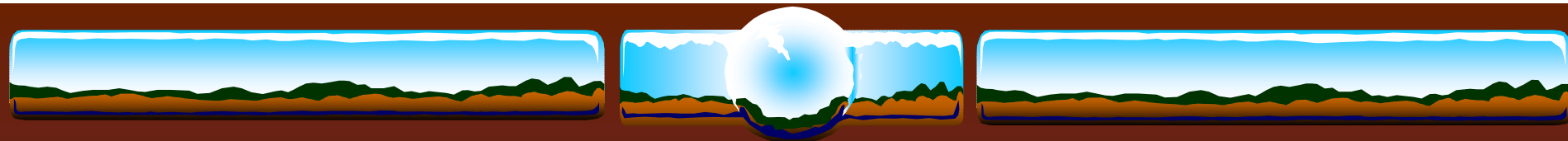
Presentation made at the
Emerging Issues Conference, sponsored by the National Ground Water Association, USGS Toxic Substances Hydrology Program, US EPA National Risk Management Laboratory, and Wessex Institute of Technology, UK; Minneapolis, MN, 7-8 June 2000.





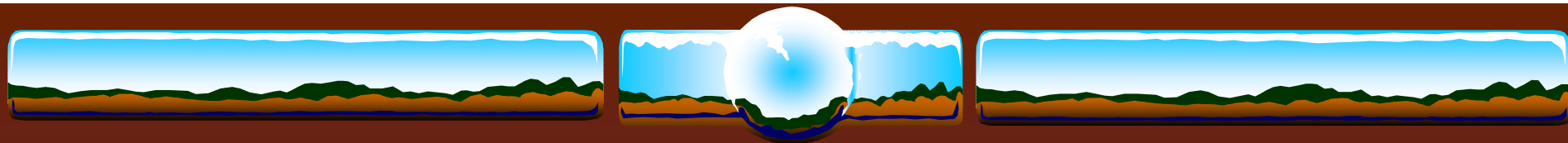
U.S. EPA Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded this research and approved the materials that formed the basis for this oral presentation. The actual presentation has not been peer reviewed by EPA.



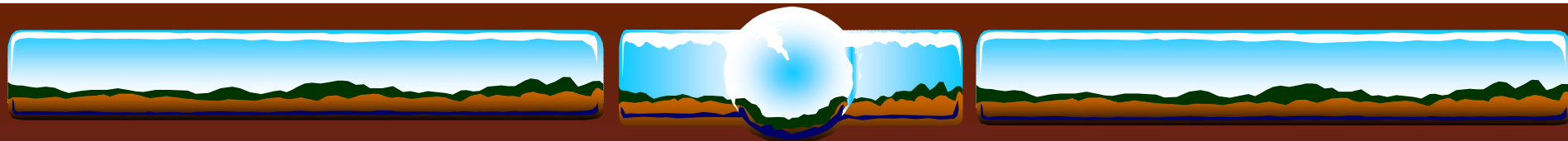
Introduction

- ❖ Information on exposure is essential for developing a quantitative health and environmental risk assessment of pharmaceuticals and personal care products (PPCPs) in the environment.
- ❖ Inherent to risk assessment is the development of state-of-the-art tools used to detect and monitor PPCPs in a variety of environmental media.



Objectives

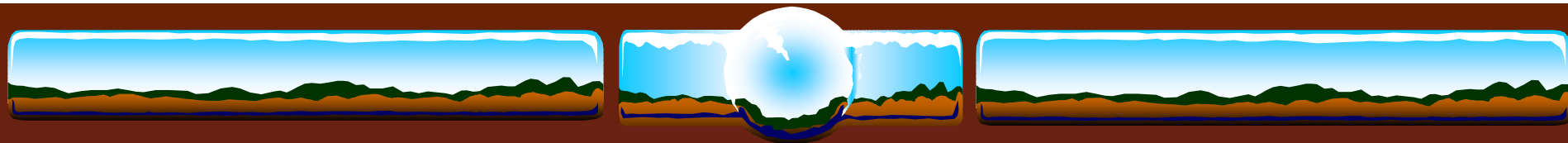
- ❖ To develop an analytical method that can detect non-volatile, polar, water-soluble pharmaceuticals in natural waters at levels that could be environmentally significant (at concentrations less than parts per billion, ppb).



Experimental

Aqueous Extractions

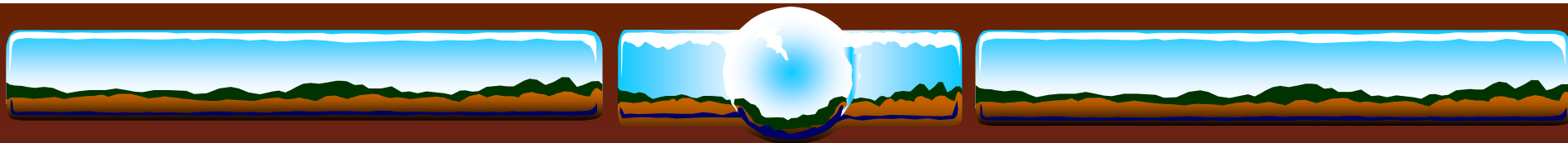
- ❖ Aqueous samples are pH adjusted to approximately pH 2.5 with 300 μL of HCl (12 N) per L of sample
- ❖ Accuprep 7000TM manifold and 48-mm nu*phase SPE C₁₈ discs (CPI International)
- ❖ Extracted with four 10-mL volumes of 99% methanol/1% acetic acid
- ❖ Evaporated to 0.1 mL using TurboVap[®] II (Zymark Corporation, Hopkinton, MA, USA)



Experimental

Chromatography

- ❖ micro-LC (liquid chromatography) columns packed in-house:
 - 160- μm i.d. (360 - μm o.d.) x 30-cm fused silica columns (Polymicro Technologies, Phoenix, AZ). Packed with 10 to 12 cm of 5 - μm ODS-Hypersil (Shandon, Astmoor, England)
- ❖ mobile phase (isocratic):
 - 80/20/1% - methanol:water:acetic acid
 - flow rates: 4 to 6 $\mu\text{L}/\text{min}$



Experimental

Mass Spectrometry

- ❖ Finnigan LCQ™, configured with an electrospray (ES) ion source:
 - positive ion mode
 - ES needle: 4.8 kV
 - 180 to 800 amu (full-scan mode, in 3 microscans with an ion injection of 200ms)



Targeted Analytes

Compound (# on RxList – 1999 http://www.rxlist.com/top200.htm)	Molecular Weight Amu	Molecular Formula
Caffeine (na)	194.19	$C_8H_{10}N_4O_2$
Fluoxetine (10)	309.33	$C_{17}H_{18}F_3NO$
Omeprazole (4)	345.42	$C_{17}H_{19}N_3O_3S$
Azithromycin (16)	749.0	$C_{38}H_{72}N_2O_{12}$
Levothyroxine Na^+ (2)	798.86	$C_{15}H_{10}I_4NNaO_4$



LODs* of Targeted Analytes

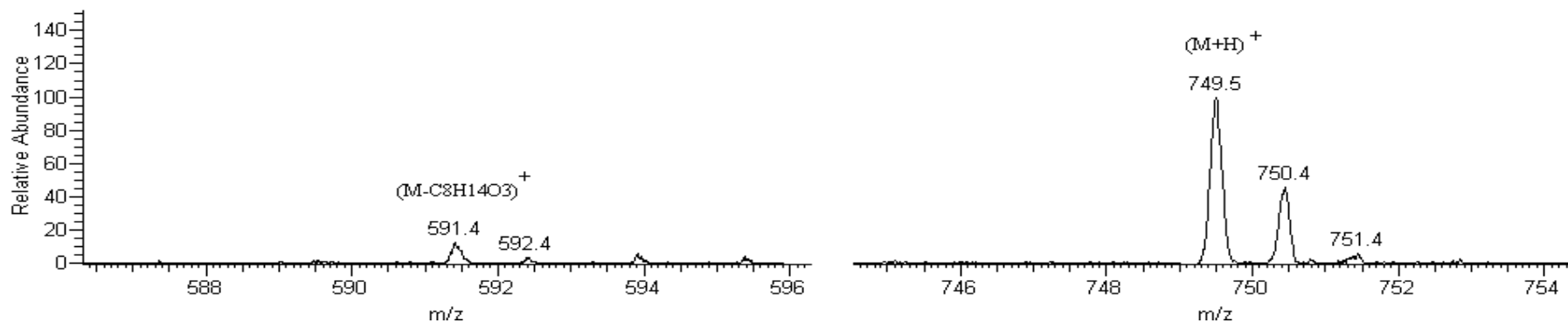
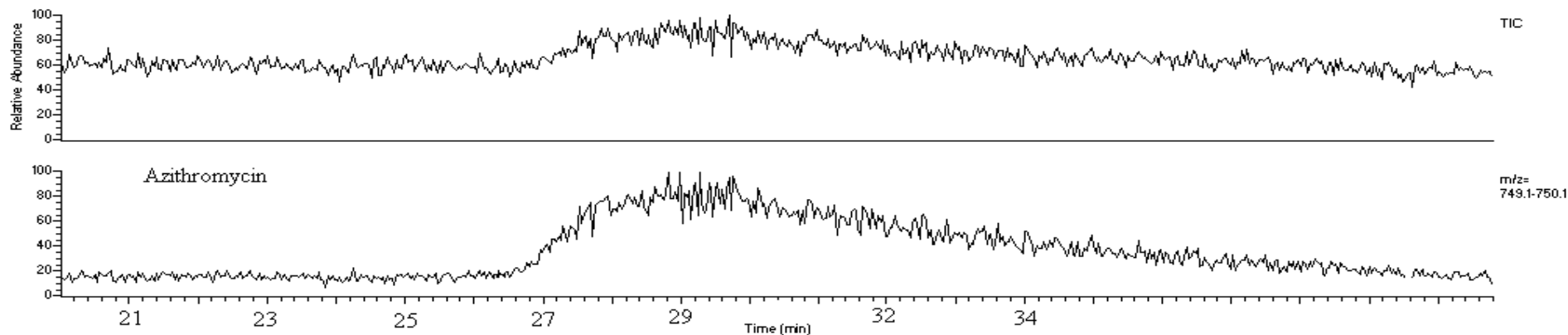
(*limits of detection)

Analyte	LOD ng
Caffeine	3
Azithromycin	4
Levothyroxine	1
Omeprazole	1
Fluoxetine	6

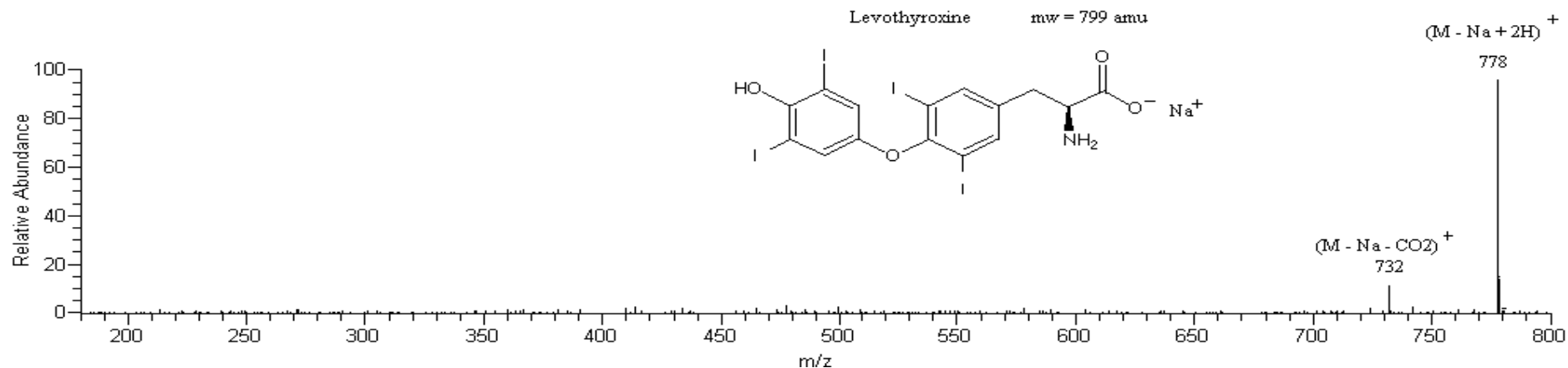
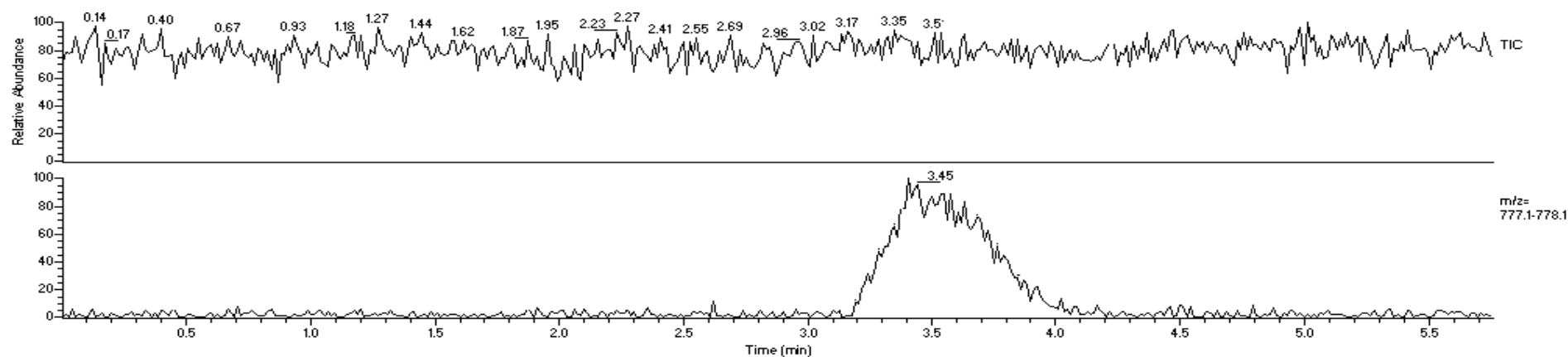


Ion Chromatogram and Mass Spectrum of Azithromycin

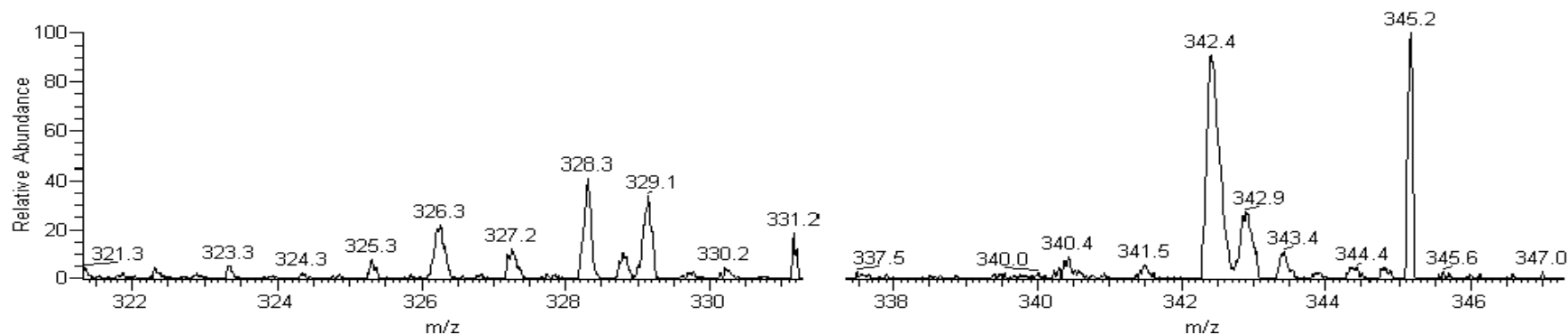
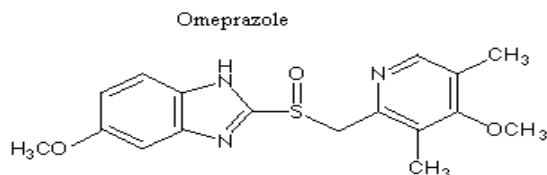
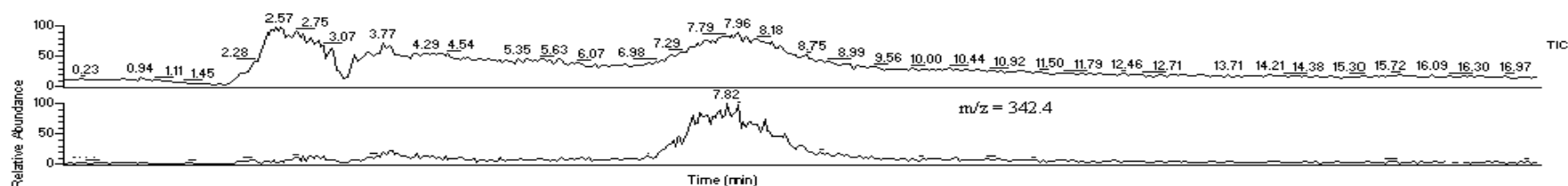
WWTP-02

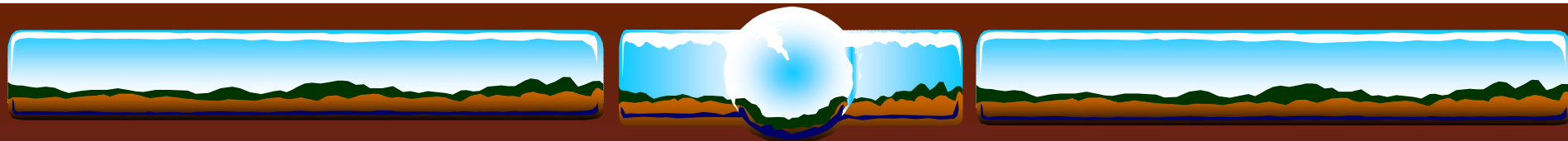


Ion Chromatogram and Mass Spectrum of Levothyroxine



Ion Chromatogram and Mass Spectrum of Omeprazole



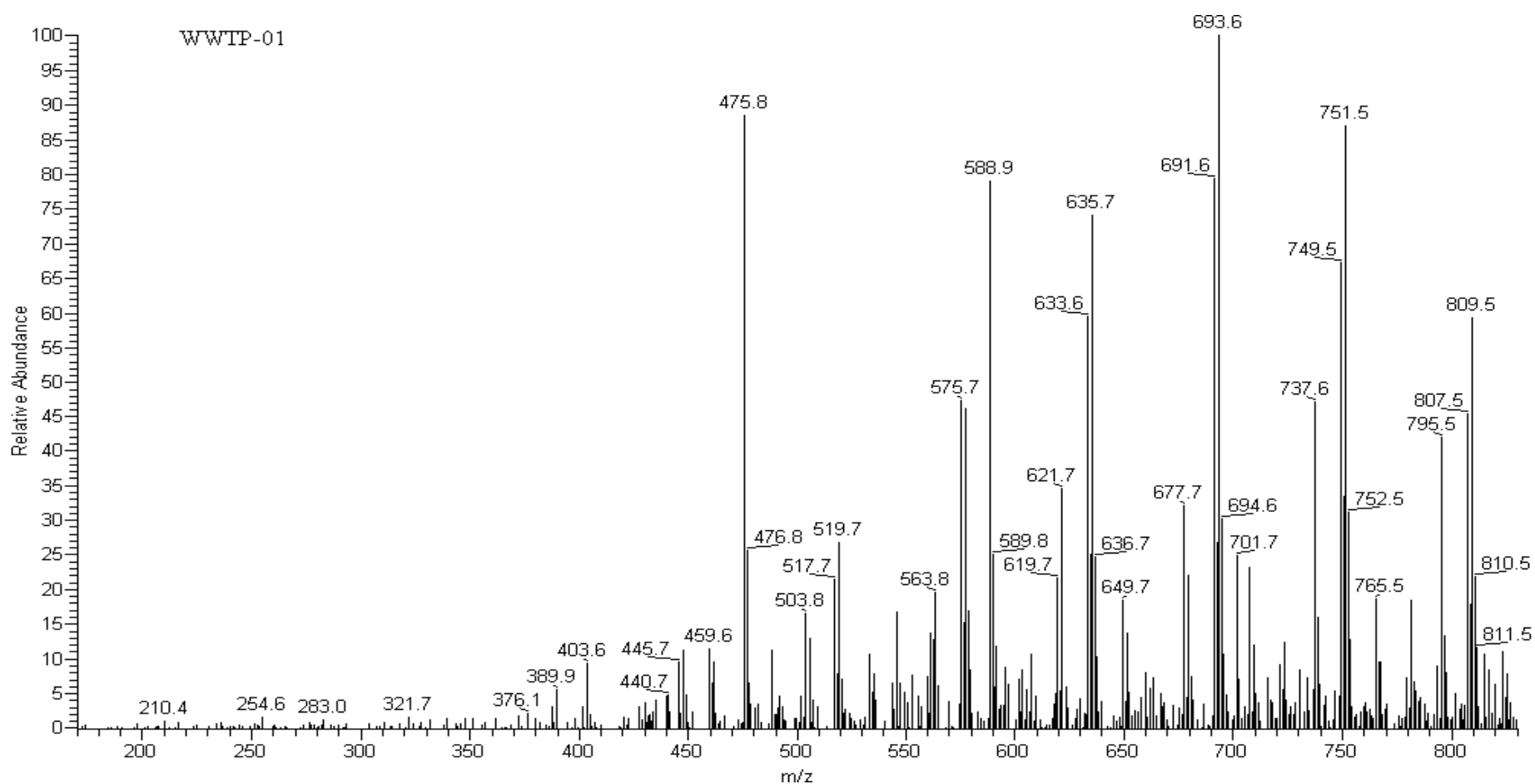


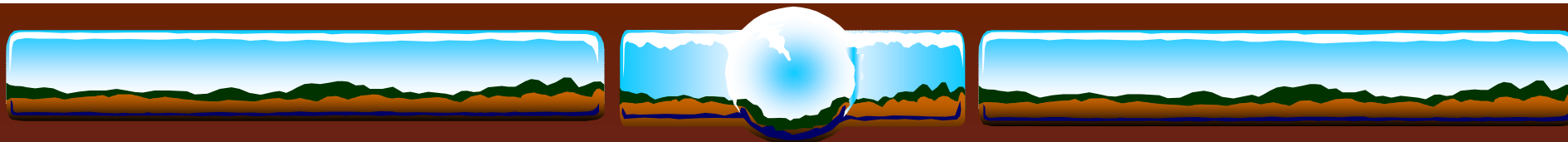
Tentatively Identified Compounds

- ❖ Other non-targeted analytes were also detected in the WWTP samples.
- ❖ For example: Polyoxypropylene glycolates (PPGs) and Polyoxyethylene glycolates (PEGs)
 - PPGs are widely used in the cosmetics industry as thickeners and in shampoo formulations
 - PEGs are widely used as additives in the food industry

Tentatively Identified Compounds

(a mixture of PPGs and PEGs)

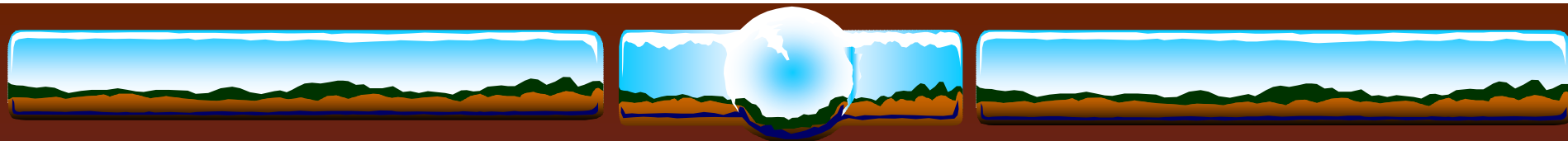




Targeted Analytes

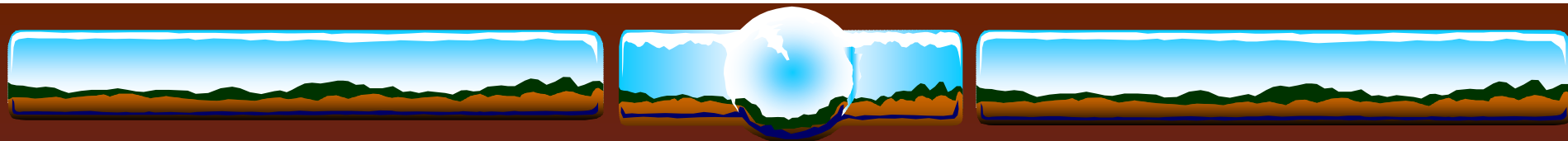
(WWTP = wastewater treatment plant)

Analytes	WWTP-01 Flow = 11 mgd < 80,000 pop.	WWTP-02 Flow = 35 mgd < 250,000 pop.
Caffeine	-	-
Azithromycin	-	+ (142 ng/L)
Levothyroxine	+	+ (940 ng/L)
Omeprazole	-	+ (133 ng/L)
Fluoxetine	-	-



Conclusions

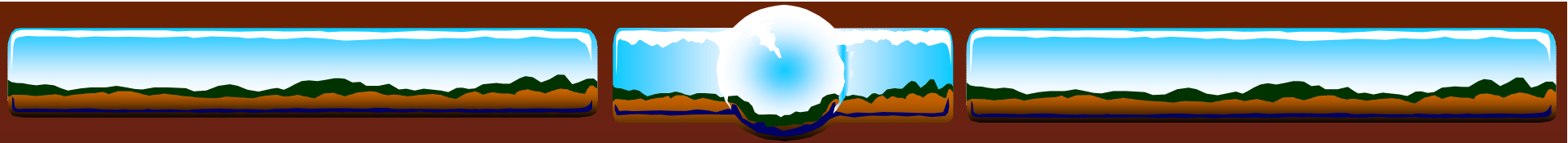
- ❖ Analytical method is sensitive. LODs \approx 1 ng for most of the targeted analytes in the LCQ ZOOM™ mode.
- ❖ Method uses “green chemistry”: < 5 mL of mobile phase per day; < 40 mLs solvent per extraction.
- ❖ Certain of the targeted pharmaceuticals are present, at low levels, in wastewater effluent (azithromycin, levothyroxine, omeprazole).
- ❖ Many other untargeted, non-volatile and polar compounds are possibly present in wastewater effluent (e.g., PPGs and PEGs).



Future Work

Environmental Sciences Division, EPA-Las Vegas

- ❖ Need to improve chromatography – addition of ion pairing reagent, e.g., ammonium acetate
- ❖ Need to improve extraction method
- ❖ Sample more WWTPs.
- ❖ Compare concentrations between influent and effluent of WWTPs.



Future Work

For Others – e.g., Toxicologists, Engineers, etc.

- ❖ Need to assess risk for both Humans and Wildlife from occurrence/exposure data as it is gathered by various scientists.
- ❖ If exposure is a risk to environmental health, then need to develop better WWTP technologies to lower the risk.
- ❖ Need to assess groundwater recharge issues.

**Prevent pollution...
It's my future, too.**

